Biological Safety Cabinets

Department of Environmental Health and Safety

Biological Safety Services

This training presents information on the design, selection, function and use of biological safety cabinets (BSC's), which are the primary means of containment developed for working safely with infectious microorganisms. Brief descriptions the facility and engineering concepts for the conduct of microbiological research are also provided. BSC's are only one part of an overall biosafety program, which requires consistent use of good microbiological practices, use of primary containment equipment and proper containment facility design.

What is Laminar Flow?

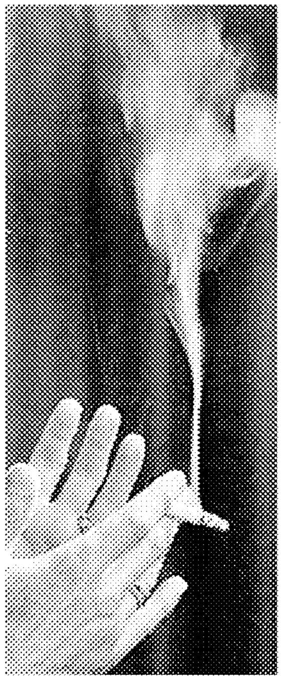
- Turn on a faucet. First very slowly, and you will see glassy, orderly flow. If there is no wind or other disturbance, nothing will change. This is called laminar flow.
- Now open the faucet to full on or watch a smoke stack. Here, the flow pattern is changing all the time.
- Turbulent flow while proceeding in a particular direction, like laminar flow, has the added complexity of random velocity fluctuations. The flow patterns never repeat themselves.

In laminar flow, sometimes called streamline flow, the velocity, pressure, and other flow properties at each point in the air remain constant.

Laminar Flow:

Turbulent flow at top

Smooth flow at bottom



When you work with equipment that uses air currents as a form of air curtain, or protection, you want the air currents to resemble the currents that are smooth and flowing together (like the smoke on the bottom of the picture) not the turbulent currents that are depicted at the top.

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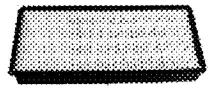
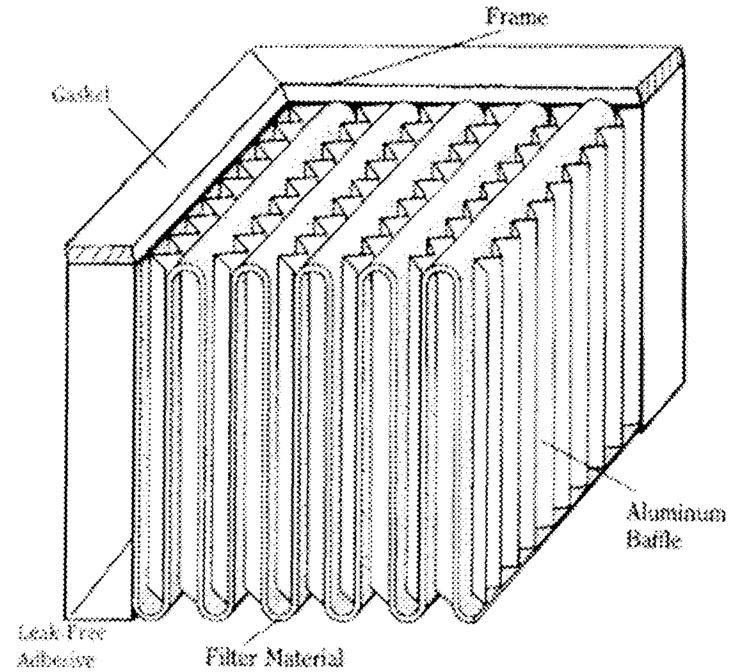
The HEPA Filter



- A common component in biosafety equipment is the HEPA filter. The HEPA filter (High Efficiency Particulate Air) removes particulates, generally called aerosols, such as micro-organisms, from the air. The HEPA filter does not remove vapors or gases. The HEPA filter can trap particles as small as .3 microns in size.
- HEPA filters are made of boron silicate microfibers formed into a flat sheet by a process similar to making paper.
- Flat filter sheets are pleated to increase the overall surface area.
- Pleats are separated by aluminum baffles which direct the airflow through the filter.

The medium of a typical HEPA filter is a single sheet of borosilicate fibers which has been treated with a wet-strength water-repellant binder. The filter medium is pleated to increase the overall surface area inside the filter frame, and the pleats are often divided by corrugated aluminum separators. The separators prevent the pleats from collapsing in the air stream and provide a path for airflow. Alternate designs providing substitutions for the aluminum separators may also be used. The filter is glued into a wood, metal or plastic frame. Careless handling of the filter (e.g., improper storage or dropping) can damage the medium at the glue joint and cause tears or shifting of the filter resulting in leaks in the medium. This is the primary reason why filter integrity must be tested when a BSC is initially installed and each time it is moved or relocated.

HEPA Filter



HEPA filters remove the most penetrating particle size (MPPS) of 0.3 µm with an efficiency of at least 99.97%. Particles both larger and smaller than the MPPS are removed with greater efficiency. Bacteria, spores and viruses are removed from the air by these filters. HEPA filter efficiency and the mechanics of particle collection by these filters have been studied and well documented therefore only a brief description is included here.

Test your Knowledge

True or False:

1. Laminar flow demonstrates smooth and orderly currents.
2. The HEPA filter does not remove vapors or gases.
3. The HEPA filter can trap particles as small as .3 microns in size.

Answers

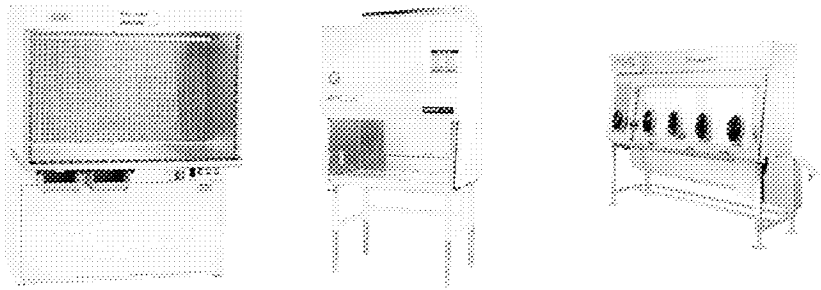
1. True. In laminar flow, sometimes called streamline flow, the velocity, pressure, and other flow properties at each point in the air remain constant. Turbulent flow while proceeding in a particular direction, like laminar flow, has the added complexity of random velocity fluctuations. The flow patterns never repeat themselves.
2. True. The HEPA filter (High Efficiency Particulate Air) removes particulates, generally called aerosols, such as micro-organisms, from the air. The HEPA filter does not remove vapors or gases.
3. True. The HEPA filter can trap particles as small as .3 microns in size.

Biosafety in Microbiological and Biomedical Laboratories (*BMBL*) 5th Ed; National Institutes of Health; Fifth Edition, Feb 2007

- Safety equipment includes BSCs, enclosed containers, and other engineering controls designed to remove or minimize exposures to hazardous biological materials. The BSC is the principal device used to provide containment of infectious splashes or aerosols generated by many microbiological procedures.

BMBL, 5th Edition

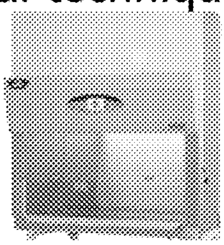
- Three types of BSCs (Class I, II, III) used in microbiological laboratories are described and illustrated in Appendix A.



Three kinds of biological safety cabinets, designated as Class I, II and III, have been developed to meet varying research and clinical needs. Most BSCs use high efficiency particulate air (HEPA) filters in the exhaust and supply systems. The exception is a Class I BSC which does not have HEPA filtered supply air.

BMBL, 5th Edition

- Open fronted Class I and Class II BSCs are primary barriers that offer significant levels of protection to laboratory personnel and to the environment when used with good microbiological techniques.



The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment.

The Class II (Types A1, A2, B1 and B2)8 BSCs provide personnel, environmental and product protection.

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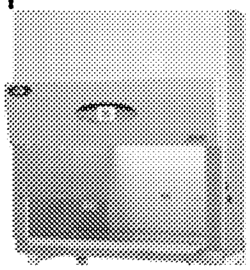
- The Class II biological safety cabinet also provides protection from external contamination of the materials (e.g., cell cultures, microbiological stocks) being manipulated inside the cabinet.



Airflow is drawn into the front grille of the cabinet, providing personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is particulate-free (environmental protection), and may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy connection. Exhaust air from Types B1 and B2 BSCs must be discharged to the outdoors via a hard connection.

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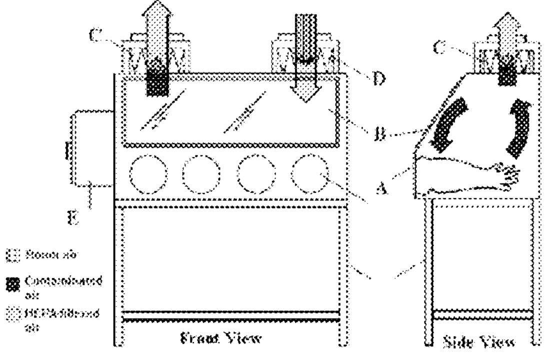
- The gas-tight Class III biological safety cabinet provides the highest attainable level of protection to personnel and the environment.

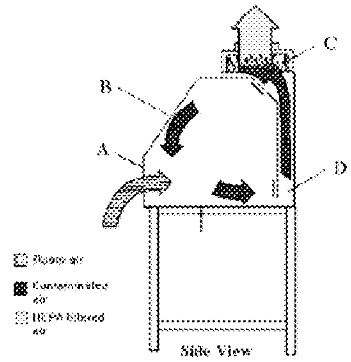


The Class III BSC (Figure 8) was designed for work with highly infectious microbiological agents and for the conduct of hazardous operations and provides maximum protection for the environment and the worker. It is a gas-tight (no leak greater than1x10-7 cc/sec of SF6 at 3 inches pressure Water Gauge14) enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank, that is accessible through the cabinet floor, or double-door pass-through box (e.g., an autoclave) that can be decontaminated
between uses. Reversing that process allows materials to be removed from the Class III BSC safely. Both supply and exhaust air are HEPA filtered on a Class III cabinet. Exhaust air must pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained by a dedicated, independent exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (minimum of 0.5 inches of pressure Water Gauge).

Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow direct manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user's direct contact with the hazardous materials. The trade-off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment. Laminar air-flow is not a characteristic of a Class III cabinet.

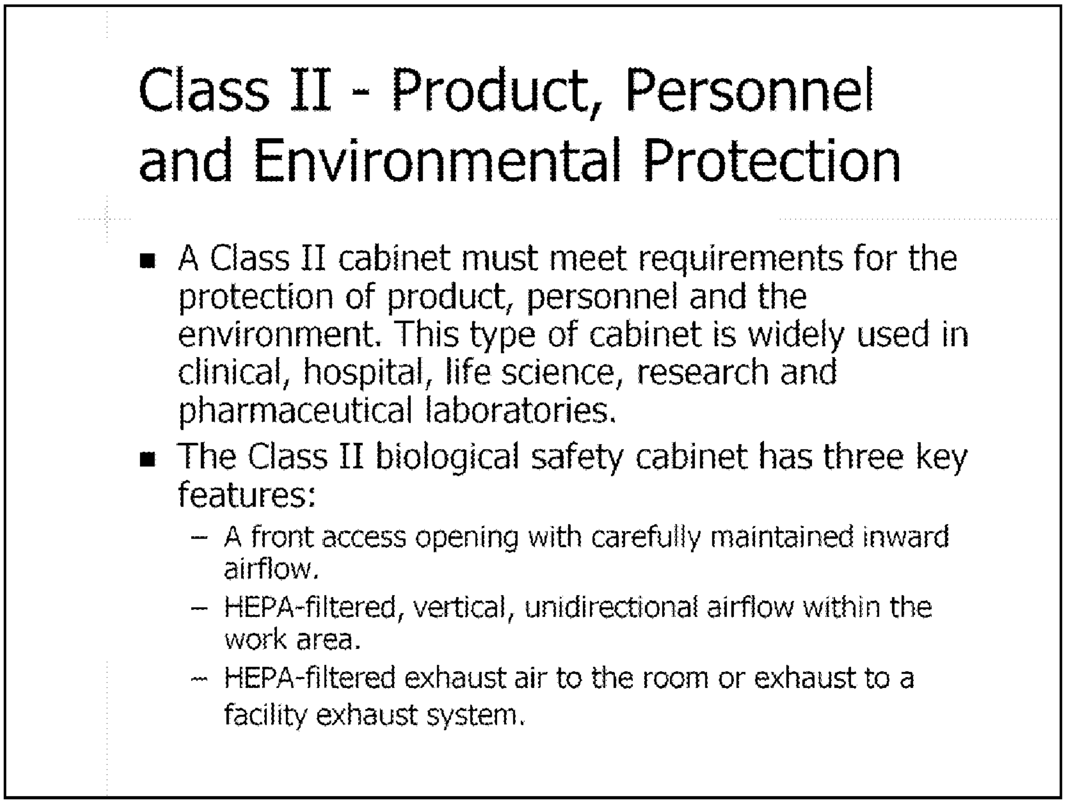
Class I - Personnel and Environmental Protection Only

- A Class I cabinet does not protect the product from contamination because "dirty" room air constantly enters the cabinet front to flow across the work surface.
- As a partial containment unit, the Class I cabinet is suitable for work involving low to moderate risk agents (biosafety levels 1,2 and 3) where there is a need for containment, but not for product protection.
- Unlike conventional fume hoods, the HEPA filter in the Class I cabinet protects the environment by filtering air before it is exhausted.
- Personnel protection is made possible by constant movement of air into the cabinet and away from the user.



In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfpm) is maintained through the front opening. Because product protection is provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases, Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures with potential to generate aerosols (e.g. cage dumping, culture aeration or tissue homogenation).

The classical Class I BSC is hard-ducted (i.e., direct connection) to the building exhaust system and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the cabinet exhaust plenum. A second HEPA filter may be installed in the terminal end of the building exhaust prior to the exhaust fan.



As biomedical researchers began to use sterile animal tissue and cell culture systems, particularly for the propagation of viruses, cabinets were needed that also provided product protection.

In the early 1960s, the "laminar flow" principle evolved. Unidirectional air moving at a fixed velocity along parallel lines was demonstrated to reduce turbulence and aid in the capture and removal of airborne contaminants from the air stream. Biocontainment technology also incorporated this laminar flow principle with the use of the HEPA filter to provide a particulate-free work environment. This combination of technologies serves to protect the laboratory worker from potentially infectious microorganisms or materials being manipulated within the cabinet and provides necessary product protection, as well. Class II BSCs are partial barrier systems that rely on the laminar movement of air to provide containment. If the air curtain is disrupted (e.g., movement of materials in and out of a cabinet, rapid or sweeping movement of the arms) the potential for contaminant release into the laboratory work environment is increased as is the risk of product contamination.

Class II Types

- Vertical, unidirectional airflow and a front access opening are common to most Class II cabinets. But, because Class II designs permit different airflow patterns, velocities, HEPA filter position, ventilation rates and exhaust methods, a sub-classification of *Type* is needed to differentiate Class II designs.
- In 2002, the National Sanitation Foundation (NSF International) restructured the Class II classification system to reflect specific performance and installation attributes.
- Class II Type A2 is the most commonly found cabinet on UMass campus.

The Class II (Types A1, A2, B1 and B2) BSCs provide personnel, environmental and product protection. Airflow is drawn into the front grille of the cabinet, providing personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is particulate-free (environmental protection), and may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged
from the building via a canopy connection. Exhaust air from Types B1 and B2 BSCs must be discharged to the outdoors via a hard connection.

NSF Classification of 2002

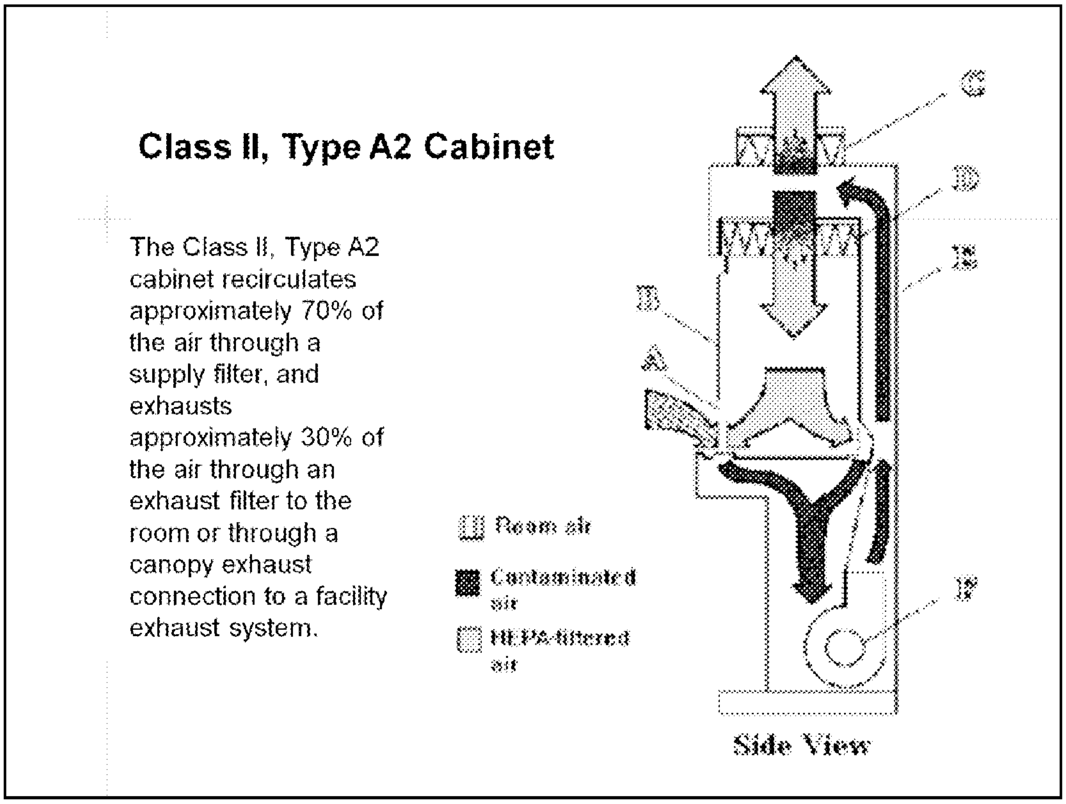
- Cabinets previously classified as Class II, Type A/B3 are now classified as Class II, Type A2. These cabinets may be exhausted to the room, or connected to a facility exhaust system via a "canopy" connection. (NSF recommends a canopy connection for cabinets exhausted to the outdoors. The Class II, Type B3 classification no longer exists for new units.
- It is important to understand differences in cabinet *Type* in order to select the proper cabinet for your application.

Environmental Health and Safety should be consulted when you are considering a purchase of new containment equipment or if you are considering a change in the risk level of the agents that you work with. EH&S will perform a risk assessment and recommend the appropriate equipment.

New NSF Classification, Adopted 2002	Previous NSF Classification	General Description
A1	Class II, Type A	▪70% air recirculated; 30% exhausted from a common plenum to the room; ▪75FPM intake; ▪may have biologically contaminated positive pressure plenum
A2	Class II, Type A/B3	▪70% air recirculated; 30% exhausted from a common plenum to the room; ▪100FPM intake; ▪biologically contaminated plenum under negative pressure or surrounded by negative pressure
A2	Class II, Type B3	▪70% air recirculated; 30% exhausted from a common plenum to a facility exhaust system; ▪100FPM intake; ▪biologically contaminated plenum under negative pressure or surrounded by negative pressure

B1	Class II, Type B1	▪40% air recirculated; 60% exhausted from cabinet; ▪exhaust air pulled through dedicated exhaust duct into facility exhaust system; ▪100FPM intake ▪all biologically contaminated plenums are negative to the room or surrounded by negative pressure plenums
B2	Class, II Type B2	▪0% air recirculated; 100% exhausted from cabinet ▪exhaust air pulled through dedicated exhaust duct into facility exhaust system; ▪100FPM intake ▪all ducts and plenums are under negative pressure ▪all contaminated ducts are under negative pressure or surrounded by directly exhausted negative pressure ducts or plenums

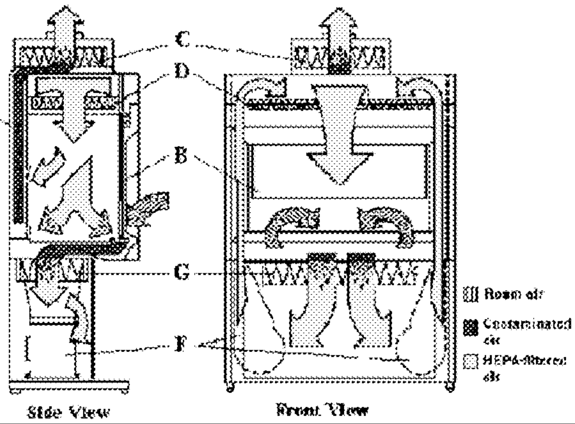
Class II Protection	From Particulates	From Vapors and Gases
Type A1 Type A2	personnel, work area (products) and environment	if exhausted to room: none; not for use with vapors and gases if exhausted to facility exhaust system, protects personnel if exhausted to a treated facility exhaust system protects personnel, the work area and the environment
Type B1	personnel, work area (products) and environment	offers more protection to personnel and the work area the closer the vapor source is located toward rear of work area; (offers protection to the environment if exhausted to treated system)
Type B2	personnel, work area (products) and environment	offers protection to personnel; (offers protection to environment if exhausted to treated system)



The Type A2 cabinet has a minimum calculated or measured inflow velocity of 100 lfpm. All positive pressure biologically contaminated plenums within the cabinet are surrounded by a negative air pressure plenum thus ensuring that any leakage from a contaminated plenum will be drawn into the cabinet and not released to the environment.

Class II, Type B1 Cabinet

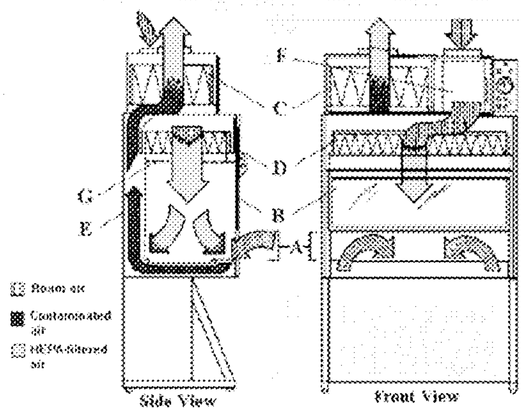
- Offers products, personnel and environmental protection.
- The cabinet exhausts approximately 60% of the circulated air through a HEPA exhaust filter.
- The remaining 40% of the air is recirculated to the work area through a HEPA supply filter.
- HEPA-filtered downflow air comprised of uncontaminated recirculated inflow air.
- Exhausts most of contaminated downflow air through a dedicated duct exhausted to the outside after passing through a HEPA filter.
- All biologically contaminated ducts and plenums are under negative pressure or surrounded by negative pressure ducts and plenums.
- Type B1 cabinets must be hard connected to an exhaust system.



Type B1 cabinets must be hard-ducted, preferably to a dedicated, independent exhaust system, or to a properly-designed laboratory building exhaust. Fans for laboratory exhaust systems should be located at the terminal end of the duct work. A failure in the building exhaust system may not be apparent to the user, as the supply blowers in the cabinet will continue to operate. A pressure-independent monitor and alarm should be installed to provide warning and shut off the BSC supply fan, should failure in exhaust airflow occur. Since this feature is not supplied by all cabinet manufacturers, it is prudent to install a sensor such as a flow monitor and alarm in the exhaust system as necessary. To maintain critical operations, laboratories using Type B1 BSCs should connect the exhaust blower to the emergency power supply.

Class II, Type B2

- Class II, Type B2 cabinets are total exhaust cabinets, widely used in toxicology laboratories and similar applications where chemical effluent is present and clean air is essential.
- There is no recirculation within the work area.
- Room air enters through a blower/motor in the top of the cabinet and passes through a HEPA supply filter into the work area as the vertical unidirectional airflow.
- Descending air is pulled through the base of the cabinet through the perforated front and rear grilles.
- Simultaneously, air entering through the perforated front opening is pulled through the grille and exhausted immediately.
- 100% of the air is pulled into the facility exhaust system for appropriate treatment.
- Type B2 cabinets must be hard-connected to an exhaust system.



This BSC is a total-exhaust cabinet; no air is recirculated within it. This cabinet provides simultaneous primary biological and chemical containment. Consideration must be given to the chemicals used in BSCs as some
chemicals can destroy the filter medium, housings and/or gaskets causing loss of containment. The supply blower draws either room or outside air in at the top of the cabinet, passes it through a HEPA filter and down into the work area of the cabinet. The building exhaust system draws air through both the rear and front grills, capturing the supply air plus the additional amount of room air needed to produce a minimum calculated or measured inflow face velocity of 100 lfpm. All air entering this cabinet is exhausted, and passes through a HEPA filter (and perhaps some other air-cleaning device such as a carbon filter if required for the work being performed) prior to discharge to the outside.

Class III Total Containment Cabinets

The Class III biological safety cabinet was designed for work with biosafety level 4 (BSL-4) microbiological agents, and provides maximum protection to the environment and the worker. Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and should permit replacement without compromising containment. Such cabinet lines are custom-built for the user.

Typical applications include:

- Working with emerging diseases or diseases marked for near eradication.
- Weighing and diluting chemical carcinogens.
- Working with high concentrations of low to moderate risk agents.
- Use of equipment or instrumentation generating high aerosol volumes.
- Maximum containment of highly infectious or hazardous experimental materials.
- An extra level of safety not available in Class I or II cabinets.
- Working with large amounts of low to moderate risk agents.

Several Class III BSCs can be joined together in a "line" to provide a larger work area. Such cabinet lines are custom-built; the equipment installed in the cabinet line (e.g., refrigerators, small elevators, shelves to hold small animal cage racks, microscopes, centrifuges, incubators, etc.) is generally custom-built as well.

Test Your Knowledge

1. What are three types of protection that Class II Biological Safety Cabinets offer?

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Answer:

Product, Personnel, Environmental; A Class II cabinet must meet requirements for the protection of product, personnel and the environment.

Test Your Knowledge

1. How much air is recirculated in a Type A2 Biological Safety Cabinet?
   A) 0% B) 40% C) 70%
2. How much air is recirculated in a Type B1 Biological Safety Cabinet?
   A) 0% B) 40% C) 70%
3. How much air is recirculated in a Type B2 Biological Safety Cabinet?
   A) 0% B) 40% C) 70%

Answers

1. C; 70% air recirculated; 30% exhausted from a common plenum to the room
2. B; 40% air recirculated; 60% exhausted from cabinet
3. A; 0% air recirculated; 100% exhausted from cabinet

Answers:

1. C; The Class III biological safety cabinet was designed for work with biosafety level 4 (BSL-4) microbiological agents, and provides maximum protection to the environment and the worker.
2. B; Class II Type A2 is the most commonly found cabinet on UMass campus.

Clean Benches

The laminar flow clean bench is a work bench or similar enclosure which has its own filtered air supply. The clean bench was developed as an adjunct to clean room technology (the need to protect the work from contamination). In recent years, the use of the clean bench has spread from research and manufacturing to other fields such as aerospace, bioscience, pharmaceutical production and food processing.

"Clean benches" are not BSCs. These pieces of equipment discharge HEPA-filtered air from the back or top of the
cabinet across the work surface and toward the user. These devices only provide product protection. They can be used for certain clean activities, such as the dust-free assembly of sterile equipment or electronic devices. Clean benches should never be used when handling cell culture materials or drug formulations, or when manipulating potentially infectious materials. The worker will be exposed to the materials being manipulated on the clean bench potentially resulting in hypersensitivity, toxicity or infection depending on the materials being handled.
"Clean benches" must never be used as a substitute for a biological safety cabinet.
Users must be aware of the differences between these two devices.

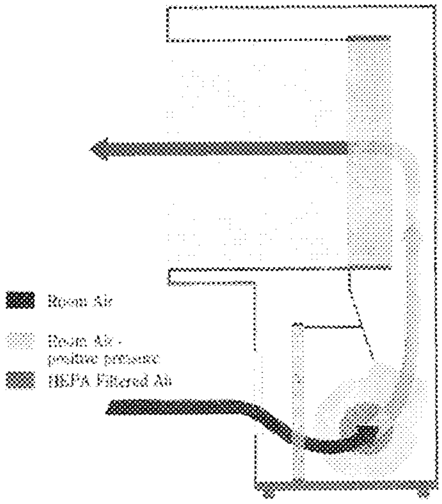
Clean Bench Function

- The clean bench provides product protection by ensuring that the work in the bench is exposed only to HEPA-filtered air.
- The clean bench is recommended for work with non-hazardous materials where clean, particle-free air quality is required.
- It does not provide protection to personnel or to the ambient environment.
- It is not designed to contain aerosols generated by the procedure; the user is exposed to these aerosols.

They may be useful, for example, in hospital pharmacies when a clean area is needed for preparation of intravenous solutions. While these units mighty have a sash, the air is usually discharged into the room under the sash, resulting in worker exposure to the material being manipulated in the enclosure. These benches should never be used for the manipulation of potentially infectious or toxic materials.

Conventional Horizontal Clean Bench

- In a horizontal laminar flow clean bench, room air is drawn into the base of the hood by the blower/motor, through a washable, reusable pre-filter.
- The air pushed up the rear plenum of the hood passes through the HEPA filter.
- Filtered air is directed horizontally across the work surface at a constant velocity of 100 FPM toward the user.



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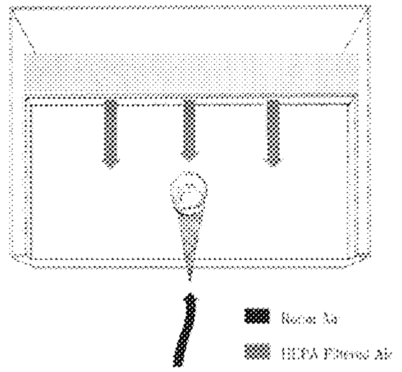
Turbulence in Clean Benches Creates Backwash

- Obstructions cause interruptions in airflow which allow particulates to enter the work area, sharply increasing particle counts inside the hood.
- Backwash is the general term applied to the entry of unfiltered room air into the work area.
- Backwash may be created when the user inserts a hand or materials into the work area.



Test Your Knowledge

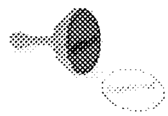
True or False:

1. The clean bench provides product protection by ensuring that the work in the bench is exposed only to HEPA-filtered air.
2. Backwash is the general term applied to the entry of unfiltered room air into the work area.
3. The clean bench is recommended for work with non-hazardous materials where clean, particle-free air quality is required.

Answers

1. True; The clean bench provides product protection by ensuring that the work in the bench is exposed only to HEPA-filtered air. The clean bench is recommended for work with non-hazardous materials where clean, particle-free air quality is required. It does not provide protection to personnel or to the ambient environment. It is not designed to contain aerosols generated by the procedure; the user is exposed to these aerosols.
2. True; Obstructions cause interruptions in airflow which allow particulates to enter the work area, sharply increasing particle counts inside the laminar flow hood. Backwash is the general term applied to the entry of unfiltered room air into the work area. Backwash may be created when the user inserts a hand or materials into the work area.
3. True; The clean bench is recommended for work with non-hazardous materials where clean, particle-free air quality is required. It does not provide protection to personnel or to the ambient environment. It is not designed to contain aerosols generated by the procedure; the user is exposed to these aerosols.

Guidelines for Work Preparation



- Check with Biological Safety Services to make sure that you are using the right cabinet for your work
- Make sure that the cabinet has been certified within the last year
- Make sure the UV light is off whenever anyone is in the room. (If the UV light is on, keep sliding sash closed or place a shield on fixed window openings.

The ideal location for the biological safety cabinet is remote from the entry (i.e., the rear of the laboratory away from traffic), since people walking parallel to the face of a BSC can disrupt the air curtain. The air curtain created at the front of the cabinet is quite fragile, amounting to a nominal inward and downward velocity of 1 mph. Open windows, air supply registers, portable fans or laboratory equipment that creates air movement (e.g., centrifuges, vacuum pumps) should not be located near the BSC. Similarly, chemical fume hoods must not be located close to BSCs.

The operational integrity of a BSC must be validated before it is placed into service and after it has been repaired or relocated. Relocation may break the HEPA filter seals or otherwise damage the filters or the cabinet. Each BSC should be tested and certified at least annually to ensure continued, proper operation.

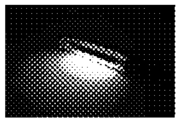
A few BSCs have UV lamps. When used, they must be tested periodically to ensure that their energy output is sufficient to kill microorganisms. The surface on the bulb should be cleaned with 70% ethanol prior to performing this test. Five minutes after the lamp has been turned on, the sensor of the UV meter is placed in the center of the work surface. The radiation output should not be less than 40 microwatts per square centimeter at a wavelength of 254 nanometers (nm). Finally, accurate test results can only be assured when the testing equipment is properly maintained and calibrated. It is appropriate to request the calibration information for the test equipment being used by the certifier. UV lights are considered ineffective and are rarely certified any longer.

A Note About UV Lights

Germicidal Benefits of UV Light in Biological Safety Cabinets

The Centers for Disease Control (CDC) and the National Institute of Health (NIH) agree that UV lamps are not recommended nor required in biological safety cabinets. UMass does not recommend UV lights in BSC's either.

Due to the short time for UV overexposure to occur, it is recommended that neither laboratory nor maintenance personnel work in a room where UV lights are on



UV light can also damage materials/equipment within or near the BSC.

UV Lights (continued)

The activity of UV lights for sterilization or decontamination purposes is limited by a number of factors including:

- Penetration - In a dynamic air stream (e.g. biological safety cabinet): UV light is not penetrating. Microorganisms beneath dust particles or beneath the work surface are not affected by the UV irradiation. UV irradiation can cause erythema that may damage both the skin and eyes of laboratory workers. Eyes and skin are primarily involved because UV does not penetrate deeply into tissue. These effects are generally not permanent but can be quite painful.

UV Lights (continued)

- Relative Humidity - Humidity adversely affects the effectiveness of UV. Above 70% relatively humidity, the germicidal effects drops off precipitously
- Temperature and Air Movement - Optimum temperature for output is 77-80°F. Temperatures below this optimum temperature result in reduced output of the germicidal wavelength. Moving air tends to cool the lamp below its optimum operating temperature and therefore results in reduced output.
- Cleanliness - UV lights should be cleaned weekly with an alcohol and water mixture as dust and dirt can block the germicidal effectiveness of the ultraviolet lights.

UV Lights (continued)

- Age – UV lamps should be checked periodically (approximately every six months) to ensure the appropriate intensity of UV light is being emitted for germicidal activity (UV C). The amount of germicidal wavelength light emitted from these bulbs decreases with age and bulb ratings (hours of use) may vary by manufacturer.
- The National Sanitation Foundation (NSF) Standard 49(6), the industry testing standard for all biohazard cabinetry, does not provide any performance criteria for UV lighting and specifically states in section 4.24.2 "UV lighting is not recommended in Class II biohazard cabinetry."

Test Your Knowledge

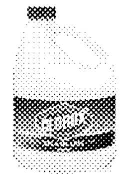
1. Which of the following groups agree that UV lamps are not recommended nor required in biological safety cabinets?
   A) CDC B) NIH C) UMass D) All of the above
2. Which of the following factors affect the decontamination effectiveness of UV lamps?
   A) Age B) Dust C) Color D) A & B only

Answers:

1. D; The Centers for Disease Control (CDC) and the National Institute of Health (NIH) agree that UV lamps are not recommended nor required in biological safety cabinets. UMass does not recommend UV lights in BSC's either.
2. D; UV lights should be cleaned weekly with an alcohol and water mixture as dust and dirt can block the germicidal effectiveness of the ultraviolet lights. UV lamps should be checked periodically (approximately every six months) to ensure the appropriate intensity of UV light is being emitted for germicidal activity (UV C). The amount of germicidal wavelength light emitted from these bulbs decreases with age and bulb ratings (hours of use) may vary by manufacturer.

Guidelines for Work Preparation (continued)

- When you first turn the cabinet on, and if you do anything to disturb the air in the cabinet, let it run for four to five minutes to filter the air and establish air flow patterns
- Disinfect the interior surface of the cabinet with the appropriate disinfectant, one that will kill any microorganisms present
- Check to be sure that the window sash is in the correct position: open 8-12 inches

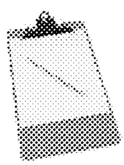


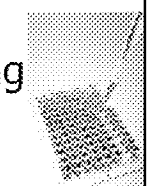
Laboratory coats should be worn buttoned over street clothing; vinyl, nitrile or other suitable gloves are worn to provide hand protection. Increasing levels of PPE can be included as determined by an individual risk assessment. For example, a solid front, back-closing laboratory gown provides better protection of personal clothing than a traditional laboratory coat and is a recommended practice at BSL-3.

Manipulation of materials should be delayed for approximately one minute after placing the hands/arms inside the cabinet. This allows the cabinet to stabilize, to "air sweep" the hands and arms, and to allow time for turbulence reduction. When the user's arms rest flatly across the front grille, occluding the grille opening, room air laden with particles may flow directly into the work area, rather than being drawn down through the front grille. Raising the arms slightly will alleviate this problem. The front grille must not be blocked with toweling,
research notes, discarded plastic wrappers, pipetting devices, etc. All operations should be performed on the work surface at least four inches in from the front grille.

Guidelines for Work Preparation (continued)

- Make sure that the drain valve is closed (if the cabinet has one)
- Have an accessible area for papers, pencils, etc., outside the cabinet so that contaminated objects are not placed inside the cabinet.
- When setting up a procedure, make sure that everything needed to complete the procedure is in the cabinet before starting



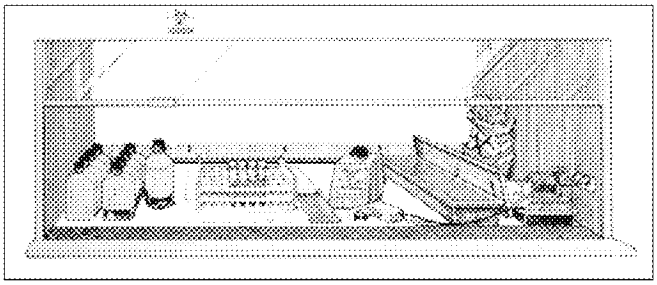


Extra supplies (e.g., additional gloves, culture plates or flasks, culture media) should be stored outside the cabinet. Only the materials and equipment required for the immediate work should be placed in the BSC.

Certain common practices interfere with the operation of the BSC. The biohazard collection bag should not be taped to the outside of the cabinet. Upright pipette collection containers should not be used in BSCs nor placed on the floor outside the cabinet. The frequent inward/outward movement needed to place objects in these containers is disruptive to the integrity of the cabinet air barrier and can compromise both personnel and product protection. Only horizontal pipette discard trays containing an appropriate chemical disinfectant should be used within the cabinet. Furthermore, potentially contaminated materials should not be brought out of the cabinet until they have been surface decontaminated. Alternatively, contaminated materials can be placed into a closable container for transfer to an incubator, autoclave or another part of the laboratory.

Guidelines for Work Preparation (continued)

- Do not block any of the grilles in the cabinet.
- Segregate clean items from contaminated items.
- Wear appropriate PPE. A long-sleeved lab coat is necessary to cover bare arms.
- Bare arms in cabinets increase particle counts like crazy!



The work flow should be from "clean to dirty“. Materials and supplies should be placed in the cabinet in such a way as to limit the movement of "dirty" items over "clean" ones. Several measures can be taken to reduce the chance for cross-contamination of materials when working in a BSC. Opened tubes or bottles should not be held in a vertical position. Investigators working with Petri dishes and tissue culture plates should hold the lid above the open sterile surface to minimize direct impaction of downward air. Bottle or tube caps should not be placed on the toweling. Items should be recapped or covered as soon as possible.

Test Your Knowledge

1. Obstructing the grilles with which objects will jeopardize containment and protection?

   A) Paper B) Metal C) Glass D) All of the above

2. After you turn the cabinet on, let it run for at least ____ minutes to filter the air and establish airflow patterns.

   A) Four B) Fifteen C) Thirty D) Sixty

3. When using a biological safety cabinet the sash should be:

   A) Wide open B) 8-12 inches from bottom

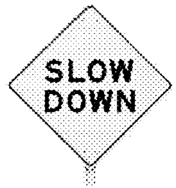
   C) Half-way open D) Totally closed

Answers:

1. D; When the user's arms rest flatly across the front grille, occluding the grille opening, room air laden with particles may flow directly into the work area, rather than being drawn down through the front grille. Raising the arms slightly will alleviate this problem. The front grille must not be blocked with toweling, research notes, discarded plastic wrappers, pipetting devices, etc.
2. A; When you first turn the cabinet on, and if you do anything to disturb the air in the cabinet, let it run for four to five minutes to filter the air and establish air flow patterns.
3. B; Check to be sure that the window sash is in the correct position: open 8-12 inches

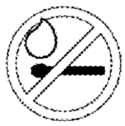
Guidelines for Working in the Cabinet

- Schedule uninterrupted work times and put a sign on the door that the cabinet is in use, as air turbulence disrupts air flow.
- Only one person should work in the cabinet at a time.
- The operator should be seated, with armpits level with the bottom of the window.
- Use good aseptic techniques.
- Use slow movements in the cabinet, minimizing entering and exiting.



Guidelines for Working in the Cabinet (continued)

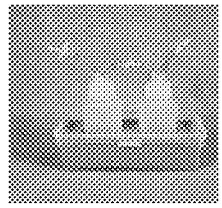
- When you do enter or exit a cabinet, do so straight on, allowing the cabinet to stabilize.
- Locate the product upstream, airflow-wise, from hands, equipment, and non-sterile items.
- Minimize movement of non-sterile items near sterile items.
- Do **NOT** use an open flame in the cabinet.

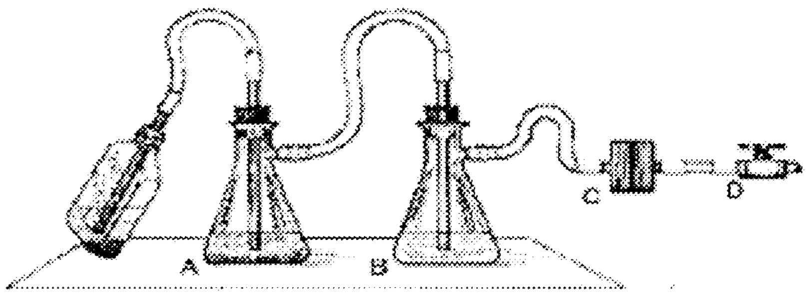


Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air being supplied to the work surface. When deemed absolutely necessary, touch-plate microburners equipped with a pilot light to provide a flame on demand may be used. Internal cabinet air disturbance and heat buildup will be minimized. The burner must be turned off when work is completed. Small electric "furnaces" are available for decontaminating bacteriological loops and needles and are preferable to an open flame inside the BSC. Disposable or recyclable sterile loops should be used whenever possible.

Guidelines for Working in the Cabinet (continued)

- Protect your vacuum system
- Use a HEPA filter

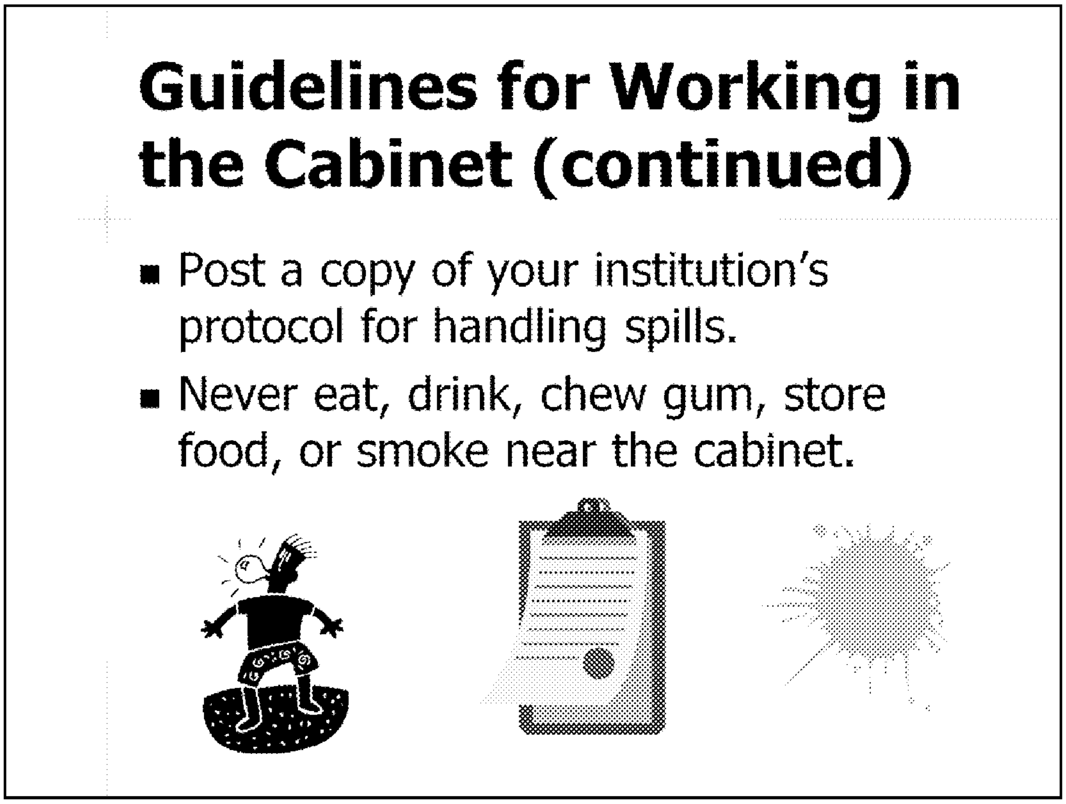




One method to protect a house vacuum system during aspiration of infectious fluids. The left suction flask (A) is used to collect the contaminated fluids into a suitable decontamination solution; the right flask serves as a fluid overflow collection vessel. An in-line HEPA filter (C) is used to protect the vacuum system (D) from aerosolized microorganisms. The in-line HEPA filter should be as near as practical to the service cock.

The aerosol/fluid trap consists of two vacuum flasks, preferably autoclavable plastic, (size dependent on amount of fluid that may accidentally be aspirated out of the collection flask), thick walled plastic tubing (to prevent tubing collapse), rubber stoppers, a filter (prevents unwanted potentially biohazardous fluid and aerosols from entering vacuum systems), and a ceramic splarger (ceramic fish tank bubbler, or piece of pipette) immersed in disinfectant. The splarger disperses aerosols passing out of the collection flask into small bubbles so that adequate contact is made with a disinfectant solution. Use an appropriate disinfectant solution (such as bleach) shown to be effective on the biohazardous material under study. Add the chemical disinfectant to the collection flasks in full strength. Allow the aspirated fluids to complete the dilution. (For example: Start with 100ml bleach, aspirate 900ml fluids and discard). The two vacuum flasks (labeled with biohazard stickers) must be placed in secondary containment such as a plastic tub large enough to contain both vessels. The tubing to the vessels should be neat and not constitute a hazard in and of itself.

These flasks should be emptied before they are 2/3 full. Replace the vacuum filter when it is clogged or if liquid makes contact with the filter. Check that all connections or seals are tight to assure the vacuum is adequate.



Spills: SOP will spell out PPE, absorbent material, disinfectant, and disposal methods.

Ingestion of pathological agents is avoived by the prevention of eating, drinking, chewing gum, smoking and storing food in laboratories.

Test Your Knowledge

1. The operator should be seated with ___ level with the bottom of the view screen sash.

   A) eyes B) waist C) armpits D) nose

2. Good BSC work practices include:

   A) Only one person working in the cabinet at a time.

   B) Use aseptic techniques.

   C) Use slow movements in the cabinet, minimizing entering and exiting.

   D) All of the above

Answers:

1. C; The operator should be seated, with armpits level with the bottom of the window.
2. D; Schedule uninterrupted work times and put a sign on the door that the cabinet is in use, as air turbulence disrupts air flow. Only one person should work in the cabinet at a time. The operator should be seated, with armpits level with the bottom of the window. Use good aseptic techniques. Use slow movements in the cabinet, minimizing entering and exiting.

Test Your Knowledge

True or False:

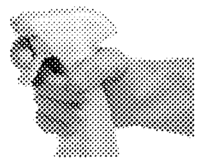
1. You are not allowed to eat or drink near biosafety cabinets, but it is ok to chew gum.
2. You should not use open flames inside Class II Type A2 biological safety cabinets.
3. Use a fast sweeping motion of the arms to enter the cabinet.
4. Minimize movement of non-sterile items near sterile items.
5. You can protect your vacuum system with a charcoal filter.

Answers:

1. False; Never eat, drink, chew gum, store food, or smoke near the cabinet.
2. True; Open flames are not required in the near microbe-free environment of a biological safety cabinet. On an open bench, flaming the neck of a culture vessel will create an upward air current which prevents microorganisms from falling into the tube or flask. An open flame in a BSC, however, creates turbulence which disrupts the pattern of HEPA-filtered air being supplied to the work surface.
3. False; Use slow movements in the cabinet, minimizing entering and exiting. When you do enter or exit a cabinet, do so straight on, allowing the cabinet to stabilize.
4. True; Locate the product upstream, airflow-wise, from hands, equipment, and non-sterile items. Minimize movement of non-sterile items near sterile items.
5. False; Protect your vacuum system by using a HEPA filter.

Guidelines for Cleanup Operations

- All equipment which has been in contact with the research agent should be enclosed, and all surfaces disinfected.
- After removing everything from the cabinet, disinfect the inner surfaces.
- Do not store equipment or supplies in or on the cabinet.

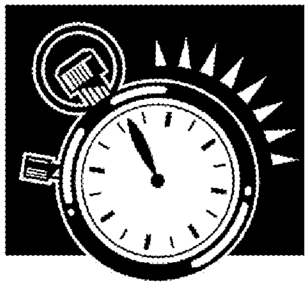


The work surface, the interior walls (*except the supply filter diffuser),* and the interior surface of the window should be wiped with 70% ethanol (EtOH), a 1:10 dilution of household bleach, or other disinfectant as determined by the investigator to meet the requirements of the particular activity. Similarly, the surfaces of all materials and containers placed into the cabinet should be wiped with 70% EtOH to reduce the introduction of contaminants to the cabinet environment. This simple step will reduce introduction of mold spores and thereby minimize contamination of cultures. Further reduction of microbial load on materials to be placed or used in BSCs may be achieved by periodic decontamination of incubators and refrigerators.

Periodic removal of the cabinet work surface and/or grilles after the completion of drain pan decontamination may be justified because of dirty drain pan surfaces and grilles, which ultimately could occlude the drain valve or block airflow. However, extreme caution should be observed on wiping these surfaces to avoid injury from broken glass that may be present and sharp metal edges. Always use disposable paper toweling and avoid applying harsh force. Wipe dirty surfaces gently. Never leave toweling on the drain pan because the paper could block the drain valve or the air passages in the cabinet.

Guidelines for Cleanup Operations (continued)

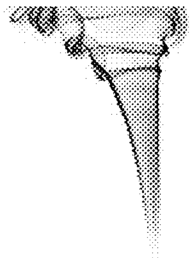
- If possible, leave the cabinet running. If it must be shut off, do so after a final purge of two to three minutes running time, then close the cabinet window completely.



BSCs are designed for 24 hours per day operation and some investigators find that continuous operation helps to control the laboratory's level of dust and other airborne particulates. Although energy conservation may suggest BSC operation only when needed, especially if the cabinet is not used routinely, room air balance is an overriding consideration.

Gas Decontamination

- When HEPA filters are changed
- Before BSC's are moved
- When internal repairs need to be done
- When BSC's are decommissioned



BSCs that have been used for work involving infectious materials must be decontaminated

before HEPA filters are changed or internal repair work is done.20-23 Before a BSC is relocated, a

risk assessment considering the agents manipulated within the BSC must be performed to

determine the need and method for decontamination. The most common decontamination

method uses formaldehyde gas, although more recently, hydrogen peroxide vapor21 and chlorine

dioxide gas have been used successfully.

Test Your Knowledge

1. When should the work surface be disinfected?
   A) Before and after every procedure
   B) Only once in the morning
   C) Only when one is going to use the cabinet
   D) Only when UV light is not working properly
2. Place the following procedure in the correct sequence with 1 as the first and 4 as the last.
   When cleaning up after completing a procedure:

- ❑ Disinfect surfaces of all equipment used
- ❑ Disinfect the BSC's inside surfaces
- ❑ Remove all items from inside the cabinet
- ❑ Place all items that may have come in contact with the agent(s), such as pipettes, in a biohazard bag or container

Answers:

1. A; Work surfaces should be disinfected before and after every procedure to avoid contamination of your work or yourself.
2. Place all items that may have come in contact with the agent(s), such as pipettes, in a biohazard bag or container
   Disinfect surfaces of all equipment used
   Remove all items from inside the cabinet
   Disinfect the BSC's inside surfaces

Test Your Knowledge

True or False

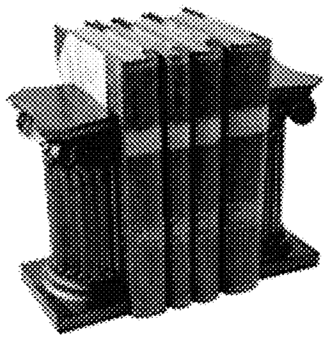
1. It is ok to store items on top of the BSC as long as they do not touch the HEPA filter.
2. If a BSC must be shut off, do so after a final purge of two to three minutes running time, then open the cabinet window completely.

Answers:

1. False. It is never ok to place materials on top of a BSC. It is easy to damage the HEPA filters and you may block air flow which will compromise the function of the BSC.
2. False. A purge of 2 to 3 minutes is sufficient but the sash should be closed completely until the BSC is used the next time.

References

- Biosafety in Microbiological and Biomedical Laboratories (*BMBL*) 5th Ed; National Institutes of Health; Fifth Edition, Feb 2007
- Safe Use of Biological Safety Cabinets; Eagleson Institute, Sanford, ME; 2002
- NSF International Standard 49;



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